in 96 well plates in DMEM/F12 supplemented with N_2. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO_2. After 2-3 days in culture, cells are fixed using 4% paraformaldehyde, and then stained using CellTracker Green CMFDA. Rho 4D2 (ascites or IgG 1:100), a monoclonal antibody directed towards the visual pigment rhodopsin is used to detect rod photoreceptor cells by indirect immunofluorescence. The results are calculated as % survival: total number of calcein - rhodopsin positive cells at 2-3 days in culture, divided by the total number of rhodopsin positive cells at time 2-3 days in culture. The total cells (fluorescent) are quantified at 20x objective magnification using a CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random.

The following polypeptides tested positive in this assay: PRO200, PRO322, PRO540, PRO846 and PRO617.

EXAMPLE 112: Ability of PRO Polypeptides to Stimulate the Release of Proteoglycans from Cartilage (Assay 97)

The ability of various PRO polypeptides to stimulate the release of proteoglycans from cartilage tissue was tested as follows.

The metacarphophalangeal joint of 4-6 month old pigs was aseptically dissected, and articular cartilage was removed by free hand slicing being careful to avoid the underlying bone. The cartilage was minced and cultured in bulk for 24 hours in a humidified atmosphere of 95% air, 5% CO_2 in serum free (SF) media (DME/F12 1:1) woth 0.1% BSA and 100U/ml penicillin and 100μg/ml streptomycin. After washing three times, approximately 100 mg of articular cartilage was aliquoted into micronics tubes and incubated for an additional 24 hours in the above SF media. PRO polypeptides were then added at 1% either alone or in combination with 18 ng/ml interleukin-1α, a known stimulator of proteoglycan release from cartilage tissue. The supernatant was then harvested and assayed for the amount of proteoglycans using the 1,9-dimethyl-methylene blue (DMB) colorimetric assay (Farndale and Buttle, Biochem. Biophys. Acta 883:173-177 (1985)). A positive result in this assay indicates that the test polypeptide will find use, for example, in the treatment of sports-related joint problems, articular cartilage defects, osteoarthritis or rheumatoid arthritis.

When various PRO polypeptides were tested in the above assay, the polypeptides demonstrated a marked ability to stimulate release of proteoglycans from cartilage tissue both basally and after stimulation with interleukin-1α and at 24 and 72 hours after treatment, thereby indicating that these PRO polypeptides are useful for stimulating proteoglycan release from cartilage tissue. As such, these PRO polypeptides are useful for the treatment of sports-related joint problems, articular cartilage defects, osteoarthritis or rheumatoid arthritis. The polypeptides testing positive in this assay are: PRO200.

EXAMPLE 113: In Vitro Antiproliferative Assay (Assay 161)

The antiproliferative activity of various PRO polypeptides was determined in the investigational, disease-oriented *in vitro* anti-cancer drug discovery assay of the National Cancer Institute (NCI), using a sulforhodamine B (SRB) dye binding assay essentially as described by Skehan et al., J. Natl. Cancer Inst. 82:1107-1112 (1990). The 60 tumor cell lines employed in this study ("the NCI panel"), as well as conditions for their maintenance and culture *in vitro* have been described by Monks et al., J. Natl. Cancer Inst. 83:757-766 (1991). The purpose

of this screen is to initially evaluate the cytotoxic and/or cytostatic activity of the test compounds against different types of tumors (Monks et al., *supra*; Boyd, Cancer: Princ. Pract. Oncol. Update 3(10):1-12 [1989]).

Cells from approximately 60 human tumor cell lines were harvested with trypsin/EDTA (Gibco), washed once, resuspended in IMEM and their viability was determined. The cell suspensions were added by pipet (100 μL volume) into separate 96-well microtiter plates. The cell density for the 6-day incubation was less than for the 2-day incubation to prevent overgrowth. Inoculates were allowed a preincubation period of 24 hours at 37°C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100 μL aliquots to the microtiter plate wells (1:2 dilution). Test compounds were evaluated at five half-log dilutions (1000 to 100,000-fold). Incubations took place for two days and six days in a 5% CO_2 atmosphere and 100% humidity.

After incubation, the medium was removed and the cells were fixed in 0.1 ml of 10% trichloroacetic acid at 40°C. The plates were rinsed five times with deionized water, dried, stained for 30 minutes with 0.1 ml of 0.4% sulforhodamine B dye (Sigma) dissolved in 1% acetic acid, rinsed four times with 1% acetic acid to remove unbound dye, dried, and the stain was extracted for five minutes with 0.1 ml of 10 mM Tris base [tris(hydroxymethyl)aminomethane], pH 10.5. The absorbance (OD) of sulforhodamine B at 492 nm was measured using a computer-interfaced, 96-well microtiter plate reader.

A test sample is considered positive if it shows at least 50% growth inhibitory effect at one or more concentrations. PRO polypeptides testing positive in this assay are shown in Table 7, where the abbreviations are as follows:

NSCL = non-small cell lung carcinoma

CNS = central nervous system

Table 7

Test compound	Tumor Cell Line Type	Cell Line Designation
PRO181	Leukemia	RPMI-8226
PRO181	NSCL	NCI-H226; NCI-H522
PR0181	Melanoma	MALME-3M; SK-MEL-5
PRO181	Ovarian	OVCAR-4
PRO181	Breast	NCI/ADR-RES
PRO181	Leukemia	MOLT-4
PRO181	NSCL	NCI-H226*
PRO181	CNS	SNB-19
PRO181	Ovarian	OVCAR-3; OVCAR-8
PRO181	Renal	A498
PRO181	Breast	MDA-MB-231/ATCC; MDA-N
PRO181	Melanoma	LOX IMVI
PRO181	Leukemia	CCRF-CEM; RPMI-8226*
PRO181	NSCL	HOP-62
PRO181	Leukemia	HL-60 (TB)
PRO237	Leukemia	K-562
PRO237	NSCL	NCI-H322M
PRO237	Colon	HCC-2998; HCT-15
PRO237	Colon	KM12
PRO237	Prostate	DU-145
PRO237	Breast	MDA-N
PRO526	NSCL	HOP-62; NCI-H322M
PRO526	Colon	HCT-116
PRO526	Melanoma	LOX IMVI; SK-MEL-2
PRO526	Ovarian	OVCAR-3
PRO526	Prostate	PC-3
PRO526	NSCL	NCI-H226
PRO526	CNS	SF-539
PRO526	Renal	CAKI-1; RXF 393
PRO362	NSCL	NCI-H322M
PRO362	Colon	HCT-116
PRO362	CNS	SF-295
PRO362	Melanoma	LOX IMVI
PRO362	Leukemia	MOLT-4; RPMI-8226; SR
PRO362	Colon	COLO 205
PRO362	Breast	HS 578T; MDA-N
PRO362	Prostate	PC-3
PRO362	Leukemia	HL-60 (TB); K-562
PRO362	NSCL	EKVX; NCI-H23
PRO362	Colon	HCC-2998
PRO362	CNS	U251
PRO362	Melanoma	UACC-257; UACC-62
PRO362	Ovarian	OVCAR-8
PRO362	Breast	T-47D
PRO362	NSCL	NCI-H522
PRO362	Renal	RXF 393; UO-31
PRO362	Breast	MDA-MB-435
PRO362	NSCL	HOP-62; NCI-H522
PRO362	Colon	KM12
PRO362	Melanoma	MALME-3M; SK-MEL-2
PRO362	Melanoma	SK-MEL-28; SK-MEL-5
PRO362	Ovarian	OVCAR-3; OVCAR-4
PRO362	Breast	MCF7
PRO866	Leukemia	HL-60 (TB); MOLT-4; SR